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Increased Cadmium Excretion Due to Oral Administration of *Lactobacillus plantarum* Strains by Regulating Enterohepatic Circulation in Mice

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Supporting Information

ABSTRACT: The heavy metal cadmium (Cd) is a contaminant widely distributed in the food chain. In the present study, 8-week oral administration of a probiotic strain, *Lactobacillus plantarum* CCFM8610, markedly decreased blood Cd levels in volunteers. Further animal study showed that three *L. plantarum* strains administered orally exhibited significantly different effects on the regulation of bile acid (BA) metabolism and Cd excretion in mice. Among the strains, *L. plantarum* CCFM8610 showed the most significant effects on enhancing hepatic BA synthesis, biliary glutathione output, and fecal BA excretion. Biliary Cd output and fecal Cd excretion were markedly increased after *L. plantarum* CCFM8610 administration, resulting in a marked reduction in tissue Cd levels. The regulation of BA homeostasis and Cd excretion was due to the suppression of the enterohepatic farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis by *L. plantarum* CCFM8610 and could be abolished by treatment with the FXR agonist GW4064. The regulatory effects were also related to the gut microbiota, as antibiotic pretreatment reversed *L. plantarum* CCFM8610-induced effects in BA and Cd metabolism.

KEYWORDS: *Lactobacillus plantarum, cadmium, enterohepatic circulation, bile acid, gut microbiota*

INTRODUCTION

Cadmium (Cd) is a heavy metal and a significant food-chain contaminant. Besides inducing pathological lesions in tissues, Cd is also a carcinogen as a number of epidemiological and clinical studies indicated increased risk of renal cancer associated with Cd exposure.[1] Several recent surveys showed that the dietary Cd intakes in adults in Europe and China were 7.6−12.0 and 15.3 μg/kg of body weight (BW)/month, respectively.[2,3] A survey based on 12 523 individuals indicated a lower average dietary Cd consumption in the U.S. population (0.54 μg/kg of body weight/week).[4] A study on 910 subjects from Southwest China showed that the dietary Cd intake of the residents in polluted areas reached 88.80−113.10 μg/kg of BW/month, indicating significant health hazards from this nonessential metal.[5] Tobacco smoking is another major source of Cd exposure.[6] Previous studies have indicated higher blood and tissue Cd contents in smokers than in nonsmokers.[7,8] It has been reported that people residing in Cd-polluted areas inhaled more than 30 μg of Cd/day from smoking locally grown tobacco.[9] On the basis of a population-based study (n = 994), the population-attributable risk of lung cancer was 73% for Cd inhalation via smoking.[10]

As a cumulative element, Cd has a biological half-life of >10 years in humans.[11] A series of long-term follow-up studies from 1979 to 2008 on a Cd-polluted hamlet in Japan showed that half-life of urinary Cd in residents was 11.4−23.4 years, and the values were affected by creatinine adjustion.[12,13] Another study indicated that the threshold safety levels of urinary Cd were 2.4 and 3.3 μg/g of creatinine in men and women, respectively.[14] The half-life of Cd is also related with the abundance of essential metals in humans, as the deficiency of iron and zinc has been reported to increase the expression of metal uptake transporters in the gut and induce a higher uptake of Cd.[15,16] Chelators are commonly used to increase Cd excretion, but they are reported to induce the loss of zinc, iron, and manganese.[17]

Our previous studies have shown that *Lactobacillus plantarum* CCFM8610 is protective against acute and chronic Cd toxicity in mice.[18,19] This probiotic is able to reduce tissue Cd accumulation and alleviate Cd-induced tissue histopathology. The protective mechanism may be due to the intestinal Cd sequestration (due to the good Cd-binding ability of this strain) and gut barrier protection of *L. plantarum* CCFM8610.[20,21] In these studies, the probiotic was used as

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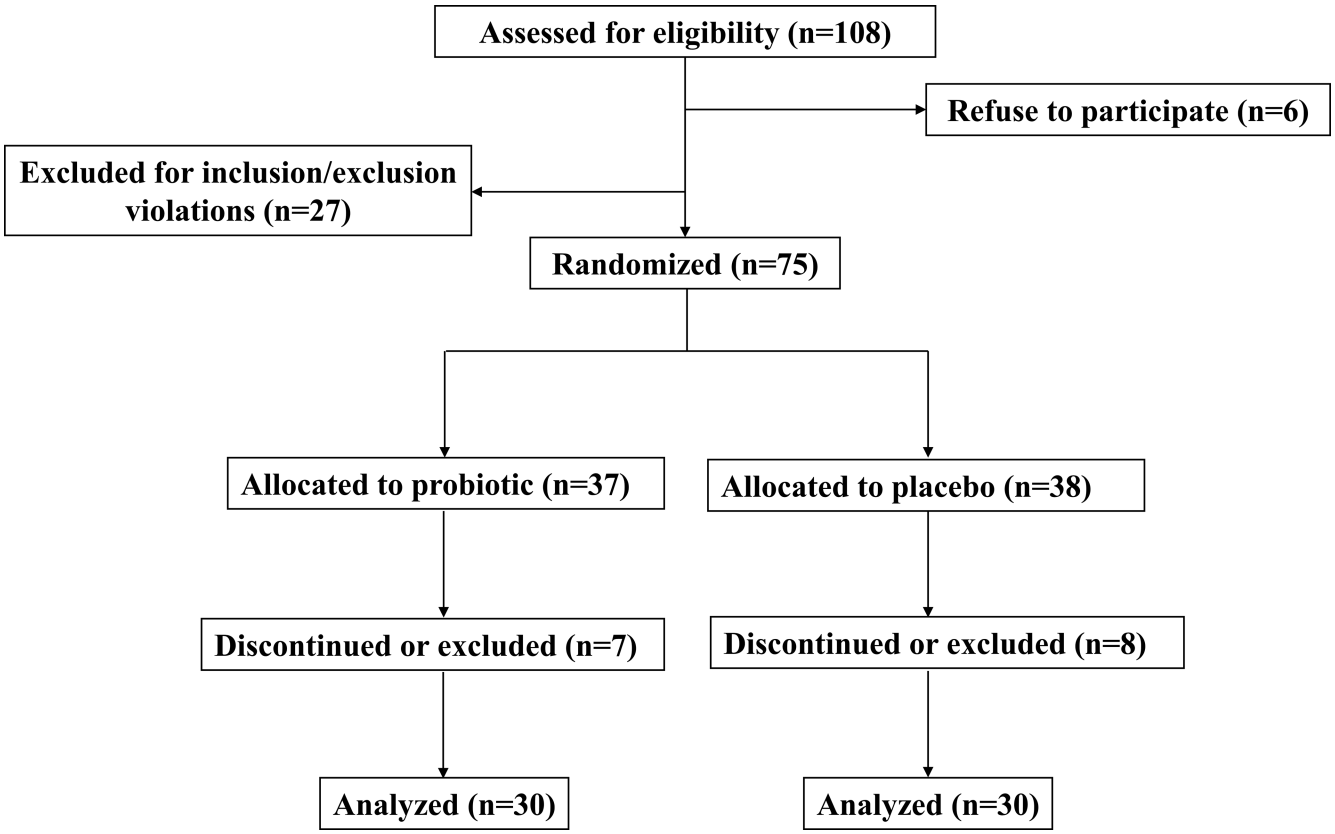

Figure 1. Consort flow diagram for the human trial.

a dietary intervention and was administered simultaneously with oral Cd exposure.

The "enterohepatic circulation" of heavy metals, including Cd, has been well-demonstrated by a number of studies. After intestinal absorption, Cd is transported to the liver, where it induces the production of metallothionein (MT) and accumulates as a Cd-MT complex.[22] The Cd-MT complex can be transported from liver to kidneys via systemic circulation and induces necrosis or apoptosis of renal tubular cells.[23] Some hepatic Cd is secreted into the intestines via bile as S-conjugates of glutathione (GSH) and cysteine and then reabsorbed by the enterocytes.[23−26] On the basis of these analyses, we hypothesized that the regulation of enterohepatic circulation may protect against Cd accumulation in the host. On the basis of a global transcriptomic analysis of the mouse liver (unpublished data), we noted that oral administration of *L. plantarum* CCFM8610 can induce gene expression changes in hepatic bile acid (BA) neosynthesis-related pathways. Some previous studies also demonstrated that *Lactobacillus* strains can modulate BA metabolism by sequestering unconjugated BAs, hydrolyzing bile salts, and regulating gut microbiota.[27−29]

In this study, three *L. plantarum* strains (CCFM8610, CCFM11, and CCFM309) with similar Cd-binding abilities[21] were used to bypass the intestinal Cd-sequestration activity of the strains. We sought to gain insight into the protective mechanisms of probiotics against Cd accumulation in the host, with a focus on their regulation of enterohepatic circulation.

MATERIALS AND METHODS

Bacterial Strains. Three *L. plantarum* strains (CCFM8610, CCFM11, and CCFM309) were obtained from the in-house Culture Collections of Food Microbiology, Jiangnan University (Wuxi, China). These strains have been previously reported to have good Cd-binding abilities in vitro.[21] For the animal experiments, the strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth (Hope Biotechnology Company, Qingdao, Shandong, China) at 37 °C for 18 h and lyophilized with skim milk as a protectant.[19] For the human trial, *L. plantarum* CCFM8610 was cultured, lyophilized, and packaged into small aluminum foil sachets by a probiotic strain manufacturer (Jiangsu Wecare Biotechnology Co., Ltd., Suzhou, Jiangsu, China).

Human Trial. The human trial followed a double-blind, placebo-controlled randomized design with two parallel arms. The project was approved by the medical ethics committee of the First People's Hospital of Guiyang City, Hunan Province, China (GYH-201801), and registered in the Chinese Clinical Trial Registry (ChiCTR1800015066). All participants received written and verbal information about the project whereupon informed consent was obtained.

A total of 108 participants were recruited from four towns in Guiyang City, Hunan province, China (Figure 1). These four towns are close to nonferrous metal-mining districts abundant in zinc, cadmium, and lead. High levels of Cd were detected in the soil (10.31 ± 0.98 Cd mg/kg) and rice (0.43 ± 0.21 Cd mg/kg) grown near the towns. The participants underwent a medical examination at the First People's Hospital of Guiyang City prior to the study. The inclusion criteria included local residents aged 45−60, with a body mass index of 18.4−28. The exclusion criteria were (1) a history of chronic disease or critical illness; (2) intake of probiotics or antibiotics during the 3 months before recruitment for the study; (3) respiratory tract infection, intestinal infection, or other diseases of the digestive tract during the previous 2 weeks; and (4) intake of any drugs during the previous 7 days.

Randomization was performed by an investigator from the First People's Hospital of Guiyang City who was blinded to the study protocol. Participants were randomly assigned to two experimental groups: a probiotic group (n = 37) and a placebo group (n = 38). The

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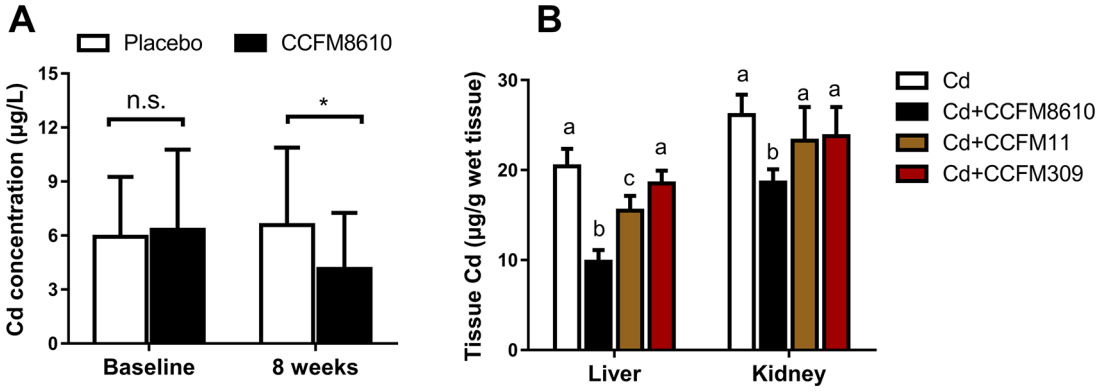

Figure 2. Effects of *L. plantarum* CCFM8610 administration on tissue and blood Cd levels in humans and mice. (A) Cd levels in the blood of volunteers during the 8-week administration of *L. plantarum* CCFM8610 (n = 30). An asterisk indicates significant differences between groups (P = 0.014). n.s. indicates no significant differences (P > 0.05) between the comparison of placebo and CCFM8610 groups. (B) Cd levels in the liver and kidneys of mice 4 weeks after Cd exposure (n = 6). For each tissue, the letters a, b, and c above the bars indicate significant differences (P < 0.05) between the groups.

participants were instructed to take one sachet of probiotic or placebo daily for 2 months. Each probiotic sachet contained *L. plantarum* CCFM8610 at a dose of 1×10^9 cfu. The placebo was identical in composition and packaging but without the addition of the bacterial strains. The sachets were packaged according to the randomization code, and the code was kept from the participants and investigators until the analyses were concluded. Fasting blood samples were collected from the participants at baseline and at the end of the study. Blood samples were stored at −80 °C for the assessment of Cd levels.

Animals and Experimental Design. Animals were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). C57BL/6 mice (male, 4−6 weeks old, weighing 14−20 g) were kept in plastic cages (5 per cage) with stainless steel grid lids in a temperature- and humidity-controlled room. The mice had free access to commercial mouse food (Xietong Organism, Inc., Nanjing, Jiangsu, China) and drinking water and were monitored every 24 h by the lab assistant. Mice were acclimatized for 7 days before the experiment. All of the protocols for the animal trials in the study were approved by the Ethics Committee of Jiangnan University, China (JN. no. 20170310-0902[25] and JN. no. 20180615c0400930[149]). The trial procedures for the care and use of experimental animals were carried out in accordance with the European Community guidelines (directive 2010/63/EU). Mice were randomly assigned to one of the following six groups and received treatments as follows.

Cd (n = 16). Mice were first given Cd-containing drinking water at 100 mg/L of $CdCl_2$ (Sinopharm Chemical Reagent Company, Shanghai, China) for 8 weeks.[19] Then the mice were given plain drinking water and received 0.5 mL of skim milk (as the vehicle control) via gavage once daily for 4 weeks.

Cd + CCFM8610 (n = 16). After an identical Cd exposure via drinking water to the Cd group for 8 weeks, mice were given plain drinking water and received an oral dose of *L. plantarum* CCFM8610 at 1×10^9 cfu each day for 4 weeks.[19]

Cd + CCFM11 (n = 6). After an identical Cd exposure via drinking water to the Cd group for 8 weeks, mice were given plain drinking water and received a single oral dose of *L. plantarum* CCFM11 at 1×10^9 cfu each day for 4 weeks.

Cd + CCFM309 (n = 6). After an identical Cd exposure via drinking water to the Cd group for 8 weeks, mice were given plain drinking water and received a single oral dose of *L. plantarum* CCFM309 at 1×10^9 cfu each day for 4 weeks.

Cd + CCFM8610 + GW4064 (n = 6). Besides an identical treatment to that of the Cd + CCFM8610 group, mice were given a gavage of a synthetic FXR agonist,[30] GW4064 (Sigma-Aldrich, St. Louis, MO, U.S.A.), at 75 mg/kg/body weight each day for the last 2 days of the experiment.[29]

Cd + Antibiotics(AN) + CCFM8610 (n = 6). Besides an identical treatment to that of the Cd + CCFM8610 group, mice were treated with a cocktail of antibiotics in drinking water, as previously described, for 3 days prior to the administration of *L. plantarum* CCFM8610.[31] During the last 4 weeks of the experiment, fecal samples were collected weekly as previously described.[20] Mice were fasted for 12 h and sacrificed under light ether anesthesia at the end of the experiment. Blood, gallbladder bile, and tissue samples were collected and stored either at −80 °C or in liquid nitrogen.

Determination of Cd Contents in Feces, Blood, Bile, and Tissues. After digestion by a Microwave Digestion System (MARS; CEM, Matthews, NC, U.S.A.), Cd levels in the samples were measured using atomic absorption spectrophotometry (Spectr AAS or AA; Varian, Palo Alto, CA, U.S.A.) or inductively coupled plasma mass spectrometry (NexIon-300X; PerkinElmer, Spokane, WA, U.S.A.).

Determination of Bile Acid Levels in Feces, Tissues, and Bile. During the last 3 days of the experiment, feces were collected according to a previous report for the analysis of fecal bile acid (BA) contents.[32] Liver, small intestine, and gallbladder bile samples were collected after the mice were sacrificed. The total contents of BA were extracted from the samples, and the levels were measured with a kit purchased from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The BA pool size was determined as the total contents of BA in the bile, liver, and small intestines.[29]

Determination of Bile Flow. Gallbladder cannulations were performed on a proportion of the animals (n = 3−5 from each group) according to a previous report at the end of the experiment.[33] Briefly, bile was collected in preweighed tubes for 30 min, and bile flow was determined gravimetrically assuming a density of 1.0 g/mL and normalized to liver weight.[34]

Determination of Glutathione in Bile. Levels of biliary glutathione (GSH) were measured with a kit purchased from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

Analysis of Real-Time Quantitative Polymerase Chain Reaction. Liver and ileum were collected and immediately stored in liquid nitrogen. Total RNA in these tissues was extracted the with Trizol reagent (Ambion, Austin, TX, U.S.A.), and NanoDrop and gel electrophoresis were used to assess RNA purity and integrity.[35] Gene expression was determined on a real-time quantitative polymerase chain reaction (PCR) system (CFX Connect; Bio-Rad, Hercules, CA, U.S.A.). The primers for the studied genes (Table S1) were selected as previously reported.[32,36] Relative quantification of the target genes was analyzed by comparison with the expression level of the *Gapdh* gene and calculated according to the $2^{-\triangle\triangle Ct}$ method.[37]

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD). Differences between means were analyzed using two-tailed Student's t tests or one-way analysis of variance, followed by Tukey's posthoc test. A P value of <0.05 was considered to be statistically significant. Statistical analyses of the obtained data were

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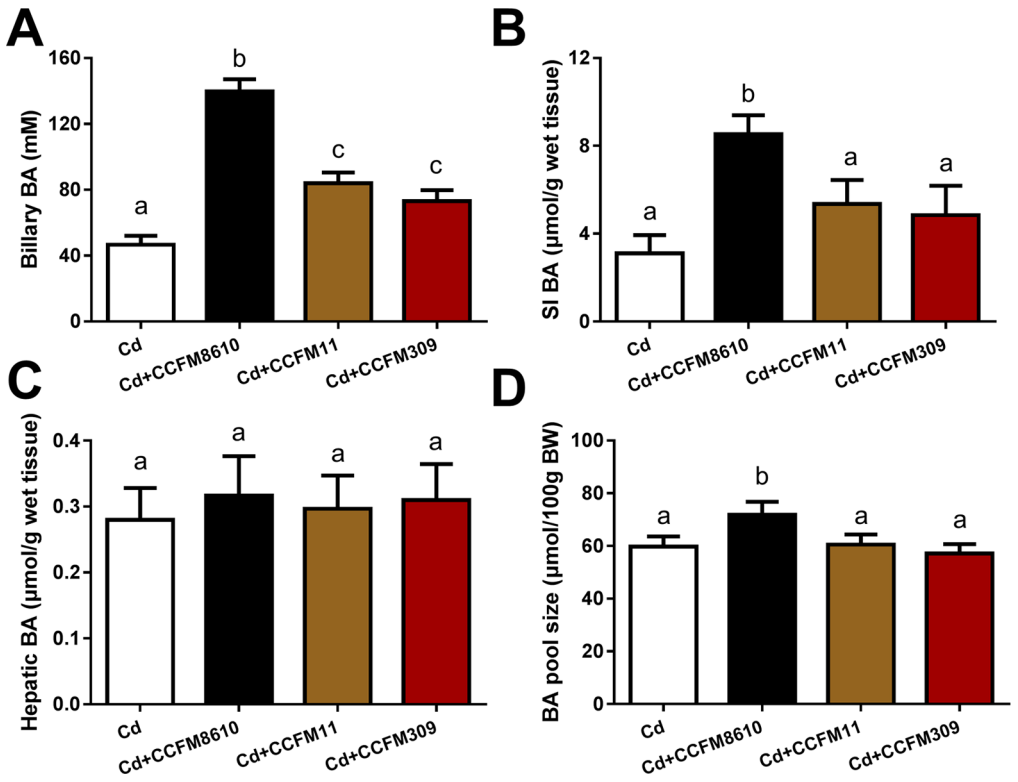

Figure 3. Effects of probiotic treatment on hepatic BA synthesis and biliary BA excretion (n = 6). (A) Biliary BA levels. (B) BA contents in the small intestines (SI). (C) BA contents in the liver. (D) BA pool size. The letters a, b, and c above the bars indicate significant differences (P < 0.05) between the groups.

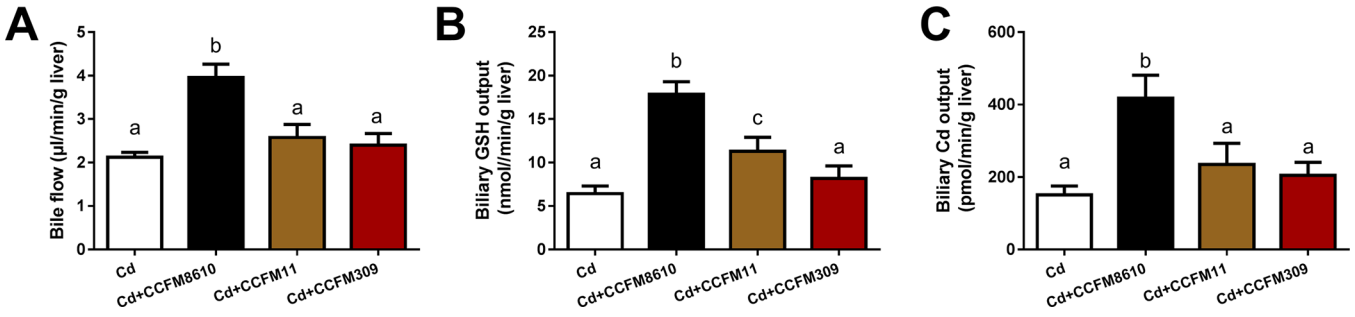

Figure 4. Effects of probiotic treatment on bile flow (A), biliary GSH output (B), and biliary Cd output (C) of mice (n = 6). The letters a, b, and c above the bars indicate significant differences (P < 0.05) between the groups.

performed and visualized using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, U.S.A.).

RESULTS

Reduced Blood Cd Levels in Humans and Decreased Tissue Cd Levels in Mice Due to *L. plantarum* CCFM8610 Administration. Seven and eight participants discontinued in probiotic and placebo groups, respectively, due to personal issues, onset of diseases, or antibiotic treatment during the trial. Therefore, a total of 60 participants completed the study (Figure 1). No significant differences in blood Cd levels were observed between the placebo and *L. plantarum* CCFM8610 groups at the baseline (Figure 2A). Eight-week oral administration of the probiotic markedly decreased blood Cd levels from 6.32 ± 4.44 to 4.15 ± 3.11 μg/L (P < 0.05), while placebo treatment did not show the same effect. As shown in Figure 2B, the hepatic Cd contents were markedly lower in the *L. plantarum* CCFM8610-treated (9.81 ± 1.30 μg/g) and CCFM11-treated (15.48 ± 1.66 μg/g) groups than that in the vehicle-treated group (20.41 ± 1.96 μg/g). *L. plantarum* CCFM8610 also reduced Cd accumulation in the kidney of mice (P < 0.05), while CCFM11 and CCFM309 failed to provide similar protection.

Increased Hepatic BA Synthesis and Biliary Output of Cd in Mice Due to by *L. plantarum* CCFM8610 Administration. The BA levels in the gallbladder and small intestines and the BA pool size in mice were upregulated by *L. plantarum* CCFM8610 administration (P < 0.05), while the hepatic BA contents remained unchanged after *L. plantarum* CCFM8610 treatment (Figure 3A–D). Along with the induction of hepatic BA synthesis, oral administration of *L. plantarum* CCFM8610 significantly upregulated bile flow and biliary GSH output (Figure 4A and B), consistent with enhanced biliary Cd excretion (P < 0.05, Figure 4C). Compared with *L. plantarum* CCFM8610 treatment, the

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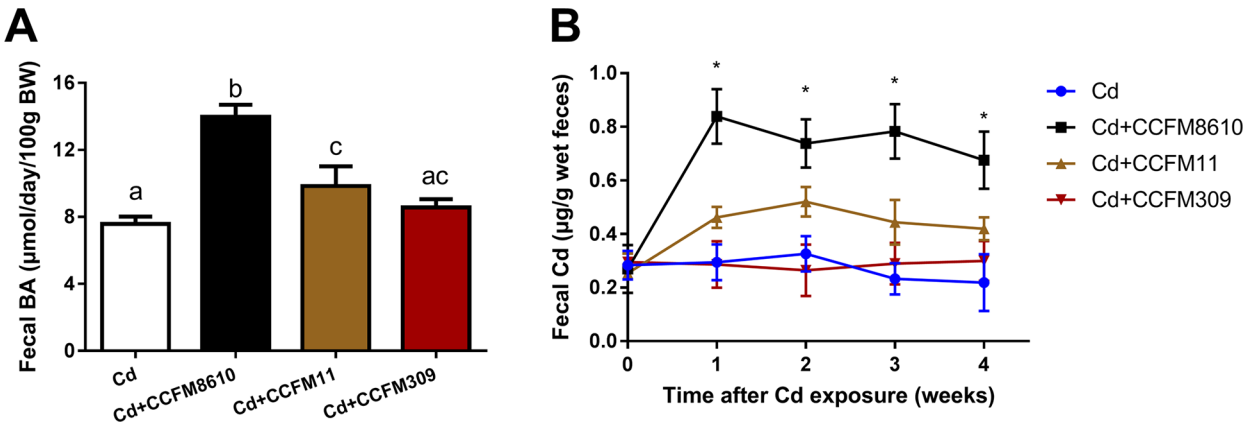

Figure 5. Effects of probiotic treatment on fecal BA and Cd excretion. (A) BA levels in feces ($n = 6$). The letters a, b, and c above the bars indicate significant differences ($P < 0.05$) between the groups. (B) Cd levels in feces. At each time point, an asterisk indicates significant differences between CCFM8610 and Cd groups ($P < 0.05$).

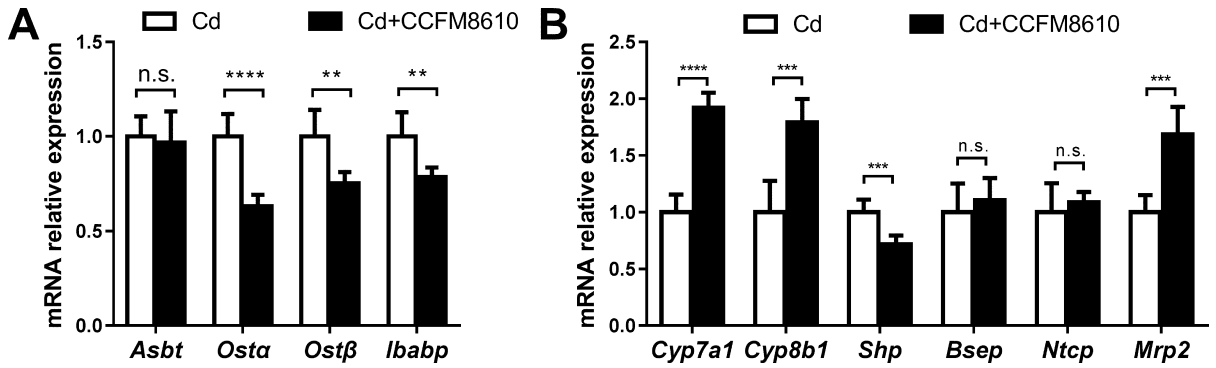

Figure 6. Effects of *L. plantarum* CCFM8610 treatment on mRNA relative expression of genes involved in BA metabolism in the ileum (A) and liver (B) of mice ($n = 6$). Data are expressed as fold change versus the Cd group (set to 1). *, **, ***, and **** indicate significant between-group differences ($P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively), and n.s. indicates no significant differences ($P > 0.05$) between groups. The full names of the genes are shown in the Results section.

other two strains had less significant effects on hepatic BA synthesis and biliary Cd excretion.

Enhanced Fecal Excretion of BA and Cd in Mice Due to *L. plantarum* CCFM8610 Administration. Compared with vehicle treatment (7.58 ± 0.43 μmol/day/100 g of body weight), fecal BA excretion was prominently enhanced after *L. plantarum* CCFM8610 treatment (13.99 ± 0.71 μmol/day/100 g body weight, $P < 0.05$, Figure 5A). *L. plantarum* CCFM8610 treatment also markedly upregulated fecal Cd levels during the experimental period ($P < 0.05$, Figure 5B). However, the *L. plantarum* CCFM11 and CCFM309 treatments failed to show similar effects on fecal excretion of BA and Cd.

Expression of Genes Involved in BA Metabolism in Mice Affected by *L. plantarum* CCFM8610 Administration. Oral administration of *L. plantarum* CCFM8610 induced lower levels of ileal mRNA expression of organic solute transporter (*Ostα/β*) and ileal BA-binding protein (*Ibabp*) than the vehicle treatment ($P < 0.05$) but exhibited no marked effects on apical sodium bile acid transporter (*Asbt*) mRNA expression (Figure 6A). *L. plantarum* CCFM8610 treatment increased the expression of cholesterol 7α-hydroxylase (*Cyp7a1*), sterol-12alpha-hydroxylase (*Cyp8b1*), and multidrug resistance-associated protein 2 (*Mrp2*) mRNA in the liver, while significantly decreasing hepatic small heterodimer partner (*Shp*) expression ($P < 0.05$, Figure 6B). The oral administration of this strain resulted in no marked alterations in bile salt export pump (*Bsep*) and sodium taurocholate cotransporting polypeptide (*Ntcp*) mRNA levels in the liver of mice ($P > 0.05$).

Effects of *L. plantarum* CCFM8610 To Induce of Cd Excretion That Were Partly Dependent on the Enterohepatic FXR-FGF15 Axis and Gut Microbiota. The ileal mRNA expression of fibroblast growth factor (*Fgf15*) was significantly inhibited after oral administration of *L. plantarum* CCFM8610, while that of farnesoid X receptor (*Fxr*) remained unaffected (Figure 7A). Co-treatment with *L. plantarum* CCFM8610 and GW4064 significantly reversed the *L. plantarum* CCFM8610-induced alterations in biliary and fecal BA levels ($P < 0.05$, Figure 7B and C). The effects of *L. plantarum* CCFM8610 on fecal Cd excretion and tissue Cd reduction were also markedly abolished by GW4064 treatment ($P < 0.05$, Figure 7D and E). The pretreatment with antibiotics increased the water intake, fecal consistency, and 120 min stool weight of mice but did not induce significant symptoms of diarrhea. Compared with *L. plantarum* CCFM8610 administration alone, antibiotic treatment significantly reversed *L. plantarum* CCFM8610-induced changes in the mRNA expression of *Cyp7a1*, *Cyp8b1*, and *Fgf15* (Figure 8A), BA levels in bile and feces (Figure 8B and C), and Cd levels in feces and tissues (Figure 8D and E).

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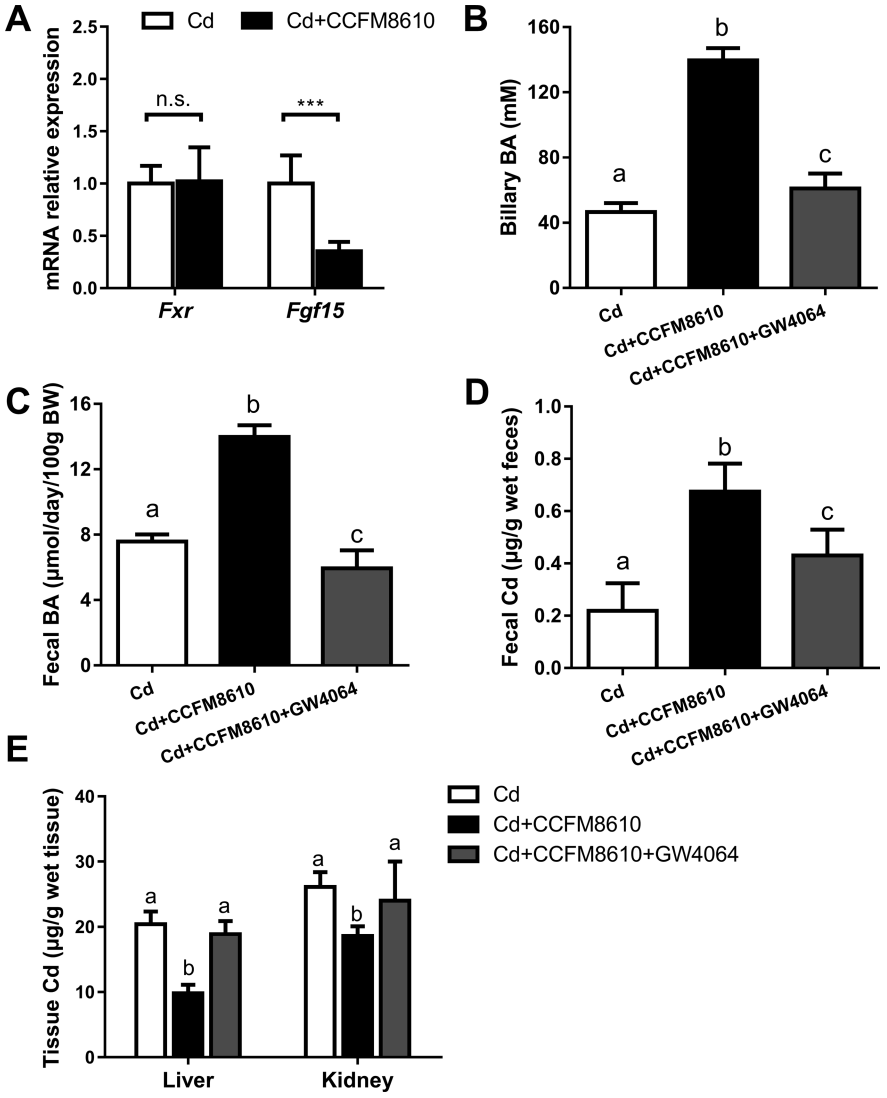

Figure 7. Role of the enterohepatic FXR-FGF15 axis in the regulation of BA homeostasis and Cd excretion by *L. plantarum* CCFM8610 administration ($n = 6$). (A) Ileal gene expression of *Fxr* and *Fgf15*. Data are expressed as fold change versus the Cd group (set to 1). *** indicates significant between-group differences ($P < 0.001$), and n.s. indicates no significant differences ($P > 0.05$) between groups. The full names of the genes are shown in the Results section. (B) BA levels in the gallbladder bile. (C) BA levels in the feces. (D) Cd levels in the feces. (E) Cd levels in the liver and kidney. The letters a, b, and c above the bars indicate significant differences ($P < 0.05$) between the groups.

DISCUSSION

The effects of probiotics against heavy metal exposure have been reported in previous in vitro, animal, and human studies.[19,38,39] Our previous studies confirmed the effects of oral *L. plantarum* CCFM8610 supplementation against intestinal Cd absorption, and the effects were mainly due to intestinal Cd sequestration and gut barrier protection.[19,20] This probiotic strain was therefore able to inhibit tissue accumulation of Cd and prevent Cd-induced tissue lesions. In this study, Cd was introduced to mice to establish a mouse model with high levels of Cd in the tissues prior to *L. plantarum* CCFM8610 treatment to investigate the potential role of *L. plantarum* CCFM8610 for treatment of Cd poisoning. The results showed that *L. plantarum* CCFM8610 was effective in decreasing the Cd levels in mice tissues (Figure 2B). As the Cd-binding ability of *L. plantarum* CCFM8610 does not play any role because the Cd has already been absorbed by the intestines and permeated the host's circulatory system, the present study indicated that the strain may possess additional properties to induce Cd excretion in mice beyond the above-mentioned protective mechanisms. This was also supported by the results from our human trial, as *L. plantarum* CCFM8610 administration significantly reduced blood Cd levels in volunteers (Figure 2A).

Enterohepatic circulation controls the storage and reabsorption of endogenous substances (such as BA and steroids) and xenobiotics (such as heavy metals and drugs) in the body.[40] Cd accumulated in the liver is released into the bile and resecreted into the gut, from where the vast majority of this toxic metal is reabsorbed and reparticipated in enterohepatic circulation.[24] We thus hypothesized that the enhancement of hepatic bile excretion and the repression of intestinal bile absorption may be useful for promoting the excretion of Cd. Previous studies have shown that probiotics are involved in the BA and cholesterol metabolism of the host.[29,41,42] These analyses led us to explore the mechanisms of *L. plantarum* CCFM8610 on Cd excretion in vivo, with a focus on its regulation of enterohepatic circulation.

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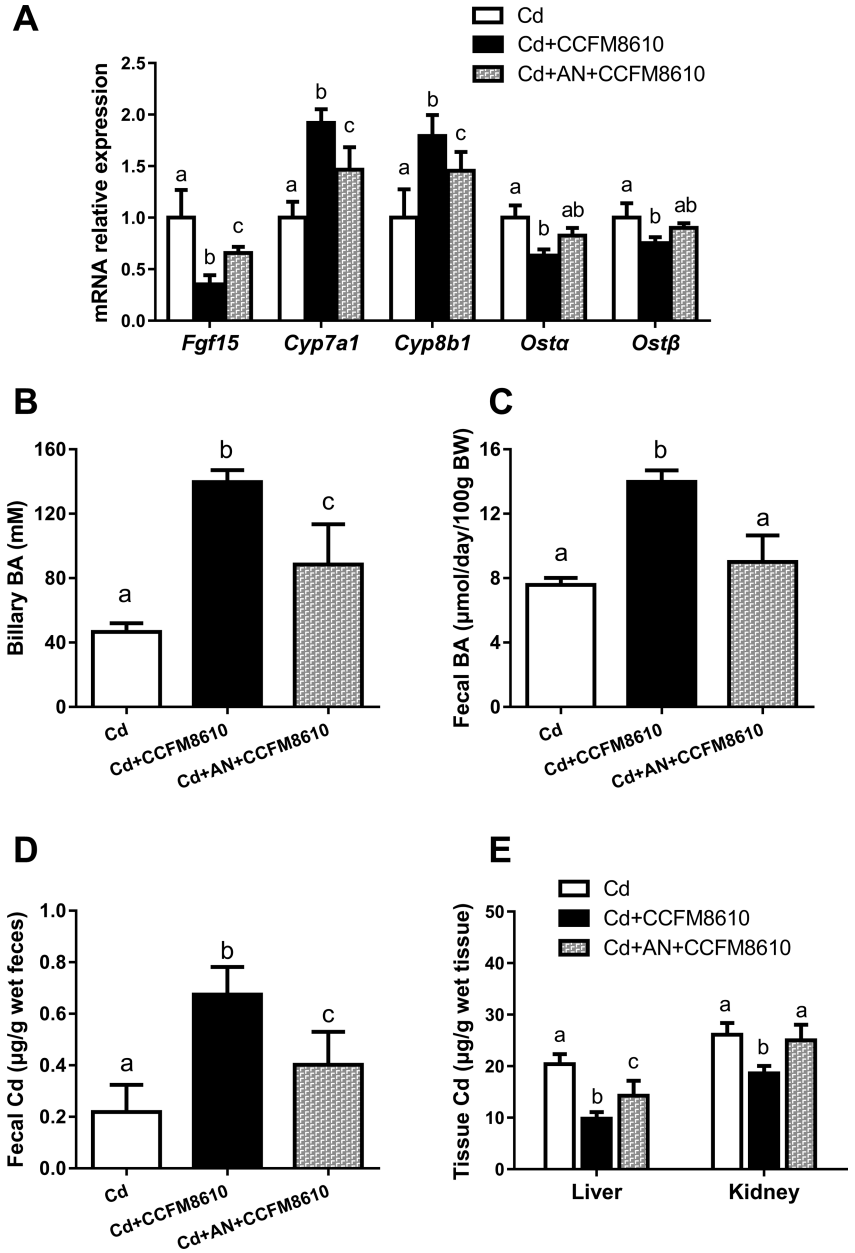

Figure 8. Role of gut microbiota in the regulation of BA homeostasis and Cd excretion by *L. plantarum* CCFM8610 administration (n = 6). (A) Expression of hepatic genes (*Cyp7a1* and *Cyp8b1*) and ileal genes (*OSTα/β* and *Fgf15*). Data are expressed as fold change versus Cd group (set to 1). (B) Biliary BA contents. (C) Fecal BA contents. (D) Fecal Cd contents. (E) Tissue Cd contents. The letters a, b, and c above the bars indicate significant differences ($P < 0.05$) between the groups. AN indicates a cocktail of antibiotics, as described in the Materials and Methods section.

The oral administration of three *L. plantarum* strains with similar Cd-binding abilities[21] produced significantly different effects on the regulation of BA enterohepatic circulation and Cd excretion, indicating that intestinal Cd sequestration is not the only protective mechanism. The strains' ability to enhance hepatic BA synthesis and bile flow was positively related to their ability to increase biliary Cd output (Figures 2 and 3). There was also a positive correlation between their ability to enhance fecal BA loss and Cd excretion (Figure 5). These results supported our hypothesis that Cd excretion can be enhanced via the regulation of enterohepatic circulation. The reduction in intestinal reabsorption of Cd could be due to excessive excretion in bile over that of ileal absorption (kinetic balance) or sequestration by the probiotic bacterium, preventing Cd from being reabsorbed. Our previous study showed that another *L. plantarum* strain, CCFM8661, could increase the fecal excretion of heavy metal lead via a similar mechanism, indicating that the regulation of enterohepatic circulation by *L. plantarum* strains is not metal-specific.[43]

On the basis of the *L. plantarum* CCFM8610-induced changes in the expression of genes involved in BA metabolism (Figure 6), the mechanism of enterohepatic circulation regulation by this strain is summarized in Figure 9. CYP7A1 and CYP8B1 are enzymes responsible for the rate-limiting step in hepatic BA biosynthesis.[44] The induction of MRP2 has been reported to increase bile flow and biliary GSH excretion.[45] Interestingly, previous studies have revealed a close coupling between the biliary secretion of Cd and that of GSH.[25] The upregulation of *Cyp7a1*, *Cyp8b1*, and *Mrp2* expression by *L. plantarum* CCFM8610 administration could therefore enhance

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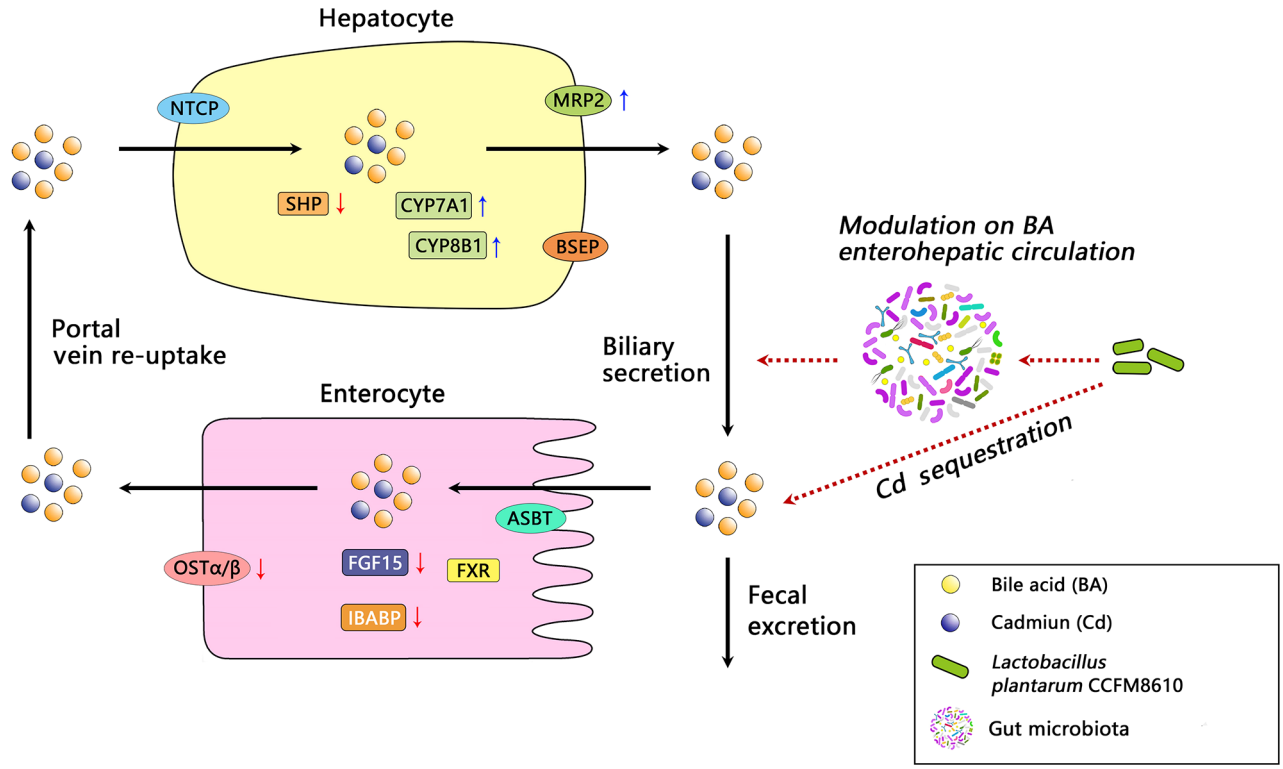

Figure 9. Proposed mechanism for the regulation of BA and Cd enterohepatic circulation by *L. plantarum* CCFM8610 administration.

the hepatic synthesis and biliary secretion of BA and Cd. In the enterocytes, oral administration of *L. plantarum* CCFM8610 exhibited no significant effects on the mRNA levels of *Asbt* but inhibited the expression of *Ibabp* and *OSTα/β* in the ileum, indicating that this strain repressed the intracellular binding and basolateral export of BA[46] rather than inhibiting ileal BA absorption.[47] With the above-mentioned regulation, *L. plantarum* CCFM8610 increased the efflux of hepatic Cd to the intestinal lumen via bile. The unabsorbed Cd was further bound by the strain in the gut and excreted via the feces. Therefore, the probiotic strain blocked the enterohepatic circulation of the toxic metal and reduced its accumulation in tissues.

The above-mentioned genes such as *OSTα/β*, *Ibabp*, *Fgf15*, *FXR*, *CYP7A1*, *CYP8B1*, and *Mrp2* are all involved in the enterohepatic FXR-FGF15 axis, a pathway that plays a role in the regulation of BA homeostasis.[48] BA-induced FXR activation induces the expression of *Fgf15*, *Ibabp*, and *OSTα/β* in the gut.[49] FGF15 in turn signals to the liver and cooperates with SHP (induced by hepatic FXR activation) to inhibit CYP7A1 and CYP8B1, thus decreasing BA synthesis.[44] *Mrp2* is also a target gene in the FXR-FGF15 axis.[50] These analyses suggest that the *L. plantarum* CCFM8610-induced modulation of biliary output and the fecal excretion of BA and Cd are related to suppression of the FXR-FGF15 axis. Supporting this hypothesis, the treatment of GW4064, a synthetic FXR agonist,[30] significantly reversed *L. plantarum* CCFM8610-induced alterations in BA metabolism and Cd excretion (Figure 7).

Previous reports have indicated an important role of gut microbiota in BA metabolism. Intestinal commensal bacteria affect BA biotransformation and directly regulate BA metabolic pathways, including the FXR-FGF15 axis.[51] In this study, mice were treated with an antibiotic cocktail to deplete the intestinal microbiota,[31] and the effects of the subsequent *L. plantarum* CCFM8610 treatment on BA and Cd metabolism were partly reversed (Figure 8). This suggests that *L. plantarum* CCFM8610 may regulate the enterohepatic circulation of BA and Cd in a gut microbiota-related manner.

In conclusion, this study confirmed that oral administration of *L. plantarum* strains can increase Cd excretion by regulating enterohepatic circulation. Among three tested strains, *L. plantarum* CCFM8610 showed the most significant effects on inducing hepatic BA synthesis, enhancing bile flow and biliary GSH output, and inhibiting the intracellular binding and basolateral export of BA in the ileum. Consistent with the regulation of BA homeostasis, biliary Cd output and fecal Cd excretion were significantly increased after *L. plantarum* CCFM8610 administration, along with a marked reduction in tissue Cd levels in mice. The above-mentioned modulation by *L. plantarum* CCFM8610 was partly dependent on enterohepatic FXR-FGF15 axis and gut microbiota.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b01004.

Primer sequences used for real-time quantitative PCR (PDF)

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Notes

The authors declare no competing financial interest.

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